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TITLE: The Effect of Recombinant Factor VIIa and Fibrinogen on Bleeding from Grade V Liver Injuries in Coagulopathic Swine

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13. ABSTRACT ***(Maximum 200 Words)***

Objectives: Recombinant factor VIIa (rFVIIa) has been used to decrease bleeding in a number of settings including hemophilia, liver transplantation, intractable bleeding and cirrhosis. This study was performed to determine if rFVIIa would reduce bleeding after a grade V liver injury in hypothermic, dilutionally coagulopathic pigs when used as an adjunct to abdominal packing and to determine the optimal dose of the drug. **Methods:** Thirty animals were randomized to receive 180 μg/kg of rFVIIa, 720 μg/kg of rFVIIa or control. After laparotomy and splenectomy animals underwent a 60% of blood volume isovolemic exchange transfusion with 5% human albumin. The animals' temperature was maintained at 33°C and a grade V liver injury was made with a clamp. Thirty seconds after injury the abdomen was packed with laparotomy sponges, resuscitation was initiated and blinded therapy was given. Animals were resuscitated to their baseline mean arterial pressure (MAP) and the study was continued for 2 hours. Serial coagulation parameters were measured at the temperature they were drawn. Following the study period, surviving animals were euthanized, post-treatment blood loss was measured and an autopsy was performed. **Results:** Ten animals were randomized to each group. Following administration of study drug, the mean prothrombin time (PT) was shorter in the treatment groups than in the control group. MAP was lower in the control group than the treatment groups throughout the study, ($p<0.01$). Mean blood loss was significantly less in the treatment groups than the control group. Mortality was not different between groups. There were no differences between the groups that received rFVIIa in any measured parameters. **Conclusions:** rFVIIa reduces blood loss in hypothermic, dilutionally coagulopathic pigs with grade V injuries when used as an adjunct to packing. Increasing the dose does not enhance the hemostatic effect.

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INTRODUCTION:

The majority of deaths following combat are secondary to hemorrhage. The goal of this research is to elucidate new methods of hemorrhage control that can be applied to the injured soldier in the field and hospital. This proposal was designed to determine the effectiveness of rFVIIa in reducing hemorrhage after a Grade V liver injury in hypothermic and coagulopathic swine and to determine if increasing the dose of rFVIIa would increase its effectiveness. We had originally intended to use rFVIIa in conjunction with fibrinogen, however our preliminary data revealed that rFVIIa was not effective in reducing blood loss after Grade V injury in swine when used as a sole agent in non-coagulopathic pigs.[1] Therefore, we chose to test its effectiveness when used as an adjunct to abdominal packing in cold, coagulopathic pigs and to determine if there was a dose response.

BODY:

Materials and Methods

Thirty Yorkshire crossbred swine, weighing approximately 30 kg, were utilized. All animals were free of disease and in apparent excellent health. Animals were allowed free access to water and to a commercial laboratory swine food. Food was held the night before the study. All animals were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care International accredited facility, and all experimental manipulations were performed in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Institutional Animal Care and Use Committee at Baylor College of Medicine.

The swine were anesthetized with an intramuscular (IM) injection of 4.4 mg/kg of Telezol. The animals were also given 0.25 mg/kg of glycopyrrolate IM. They were then intubated with a 7 mm Mallinckrodt endotracheal tube and placed on mechanical ventilation with settings of 10 ml/kg tidal volume, a rate of 12-14 breaths per minute and 100% oxygen. Anesthesia was maintained using 2% isoflurane and an esophageal temperature probe was placed.

Once the animals were fully anesthetized, cutdowns were performed and polyethylene tubing was placed in the left external jugular vein and left common carotid artery. The venous line was used for study drug infusion and fluid resuscitation. The arterial line was used for continuous blood pressure monitoring and blood sampling. Mean arterial pressure, systolic pressure, diastolic pressure and heart rate were recorded and averaged every 10 seconds using a digital data collection system with a blood pressure analyzer (Micro-Med®, Louisville, KY).

The animals underwent midline laparotomy, Foley catheter placement and splenectomy. The spleen was weighed and lactated Ringer's (LR) solution at room temperature was infused at 100 ml/min to replace 3 times the spleen weight. Animals then underwent an isovolemic, exchange transfusion with 5% human albumin at room temperature. An estimated 60% of the animals' blood volume was removed via controlled hemorrhage from the carotid arterial line. The equation blood volume (ml/kg) = $161.4751(\text{body weight}^{-0.2197})$ was used. The animals' temperature was then

standardized to an esophageal temperature of 33°C. This was done by lavaging the abdomen with room temperature lactated Ringer's solution.

Pre-weighed laparotomy pads were placed in both gutters and the pelvis to facilitate blood collection. A standardized Grade V liver injury was made with a specially designed liver clamp. For the purposes of this model, a Grade V injury is defined as an injury to a central hepatic vein. This is consistent with the definition of a Grade V injury as indicated by the American Association for the Surgery of Trauma Organ Injury Scaling system.[2]

The current animal model is based upon our experience in previous studies of hemorrhage control utilizing the Grade V liver injury model.[3-5] The clamp was positioned in the middle of the liver placing the right hepatic vein, left hepatic vein and portal vein at risk for injury. Blood loss was collected by suction. Thirty seconds after injury, blinded therapy consisting of either 180 μg/kg of rFVIIa, 720 μg/kg or the equivalent amount of buffer solution was infused. Simultaneously, the liver injury was packed with laparotomy sponges and resuscitation with lactated Ringer's solution at 100 ml/min was initiated. The temperature of the resuscitation fluid was varied to maintain a core temperature 33°C. The abdomen was then closed. Animals were resuscitated to their baseline MAP. Pre-treatment blood loss was calculated as the sum of the volume of blood suctioned and the difference in weight of the pre-weighed laparotomy pads before and after bleeding.

The study was continued for 2 hours from the time of injury. During this time period, lactated Ringer's solution was given as needed to maintain the baseline MAP. The core temperature was maintained at 33°C by varying the fluid temperature and by using a Bair Hugger warming system (Eden Prairie, MN). Time of death was recorded for those animals that did not survive the 2 hour study period.

After 2 hours, the animals were euthanized and the abdomen was opened. Free blood in the abdomen was suctioned and the packing laparotomy sponges were weighed. Post treatment blood loss was calculated as the sum of the volume of blood suctioned and the difference in weight of the laparotomy sponges from before placement to the end of the study. An autopsy of the liver was performed to insure that injuries were comparable between groups.

Laboratory studies were drawn at baseline, prior to injury, 5 minutes after injury, 1 hour after injury and at the end of the study. Laboratory studies included arterial blood gas, complete blood count, prothrombin time, partial thromboplastin time, fibrinogen, thrombin anti-thrombin complexes and d-dimers. Coagulation studies were performed at the temperature at which they were drawn.

Statistical Analysis

The paired t-test was used to compare means of matched continuous variables. Dichotomous data were analyzed with the chi-square test. If the value of a categorical variable in any cell was less than five, Fisher's exact test was utilized. Statistical analysis was performed using commercially available software from Stata Corporation, College Station, Texas. Statistical significance was defined as a p-value < 0.05.

Results

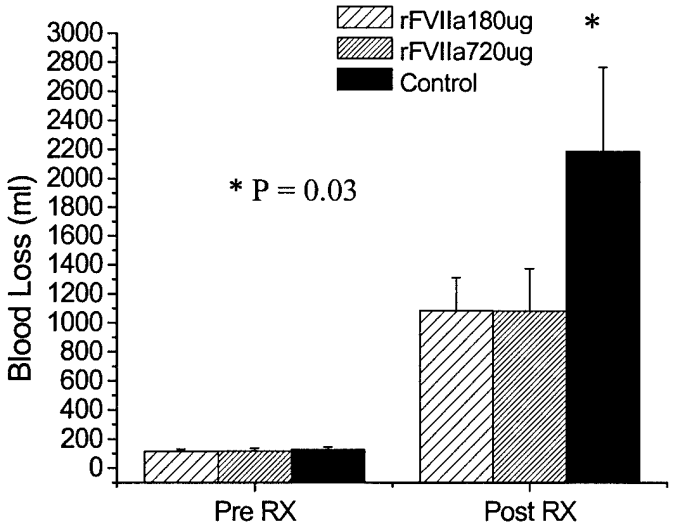
Ten animals randomized to each of the 3 groups. Mean weight, temperature, number of vessels injured and fluid resuscitation are shown in Table 1. Weight and temperature were similar between groups, as was the severity of injury. Animals in the control group received approximately twice as much resuscitation fluid as animals in the 2 treatment groups. However, due to large standard deviations, these differences were not statistically significant.

Table 1.

	RFVIIa 180 ug/kg	RFVIIa 720 ug/kg	Control	P
Mean Weight (kg)	31.4	31.7	31.6	NS
Mean Injury Temp (°C)	33.1	33.3	33.2	NS
Mean Vessels Injured	2	2.1	2.1	NS
Mean Fluid Resuscitation (ml)	2717	2734	4514	0.2

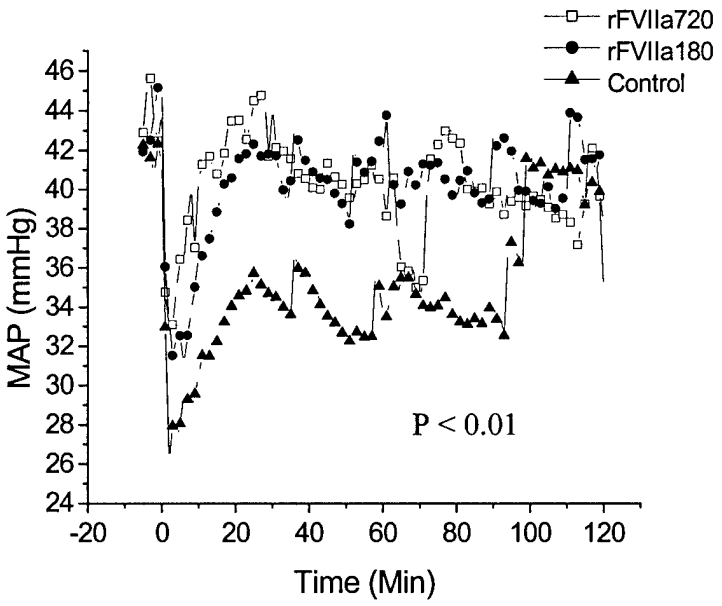
Blood loss after treatment in the control group was approximately twice that of either treatment group. This difference was significant at the $p = 0.03$ level. There was no difference in blood loss between the 2 treatment groups. This data is shown in Figure 1.

Figure 1. Blood loss pre and post treatment.



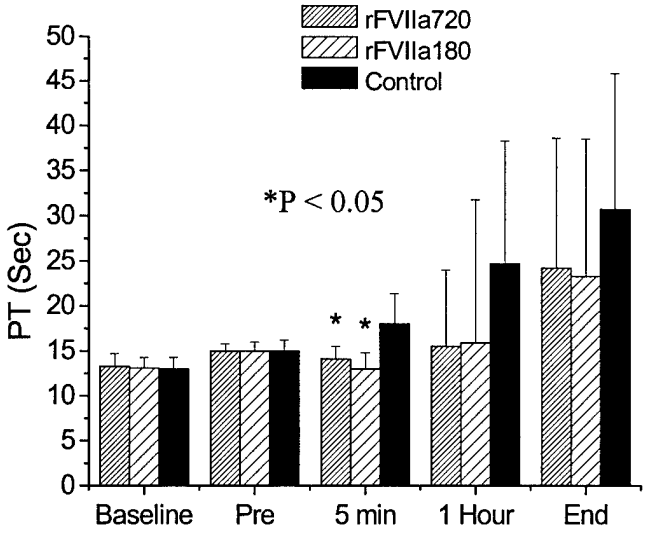
The mean arterial pressures of the 3 groups are shown in Figure 2. The control group had a low mean nadir MAP and the blood pressure of the control group was significantly lower than the treatment groups throughout the study. There was no difference between the 2 treatment groups.

Figure 2. Mean arterial pressure over the course of the study.



Mean prothrombin times measured over the course of the study are shown in Figure 3. Following treatment, there was a significant reduction in the prothrombin times in the 2 treatment groups as compared to the control group. This difference was no longer significant 1 hour after injury or at the end of the study.

Figure 3. Prothrombin time



Partial thromboplastin times were not significantly different over the course of the study between groups.

Figures 4 and 5 show thrombin antithrombin complexes (TATs) and d-dimers measured serially between groups. Both groups that received rFVIIa exhibited significantly elevated TATs compared to the control group. These differences were present 5 minutes after treatment and throughout the remainder of the study. Similarly, d-dimers were significantly elevated in the treatment groups relative to the control group. These differences were present at 1 hour and at the end of the study.

Figure 4. Thrombin antithrombin complexes

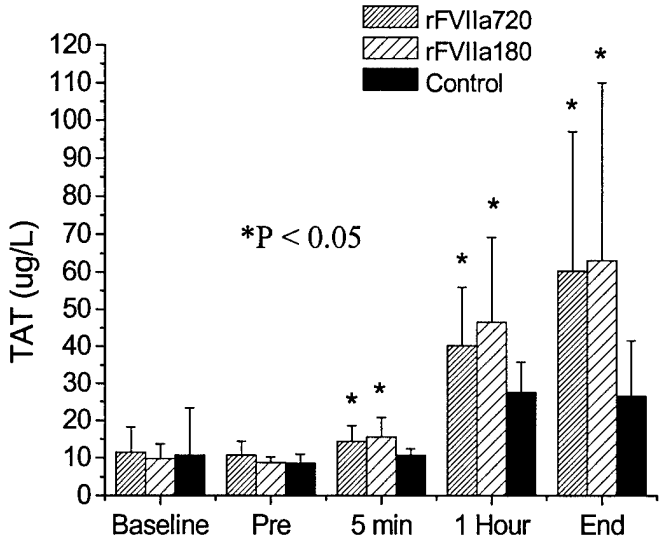
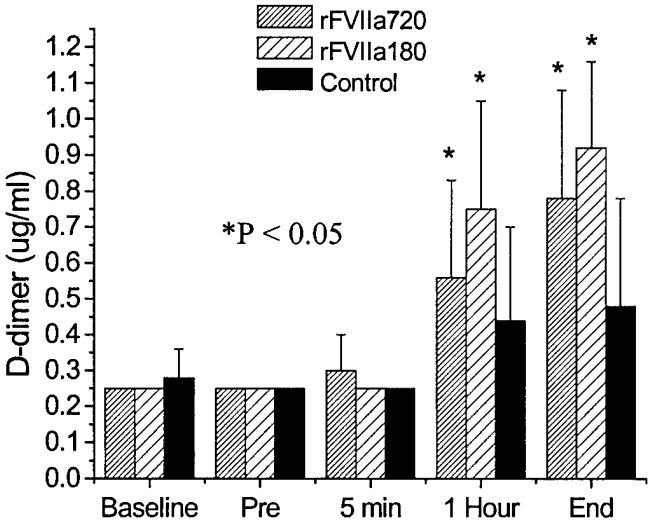


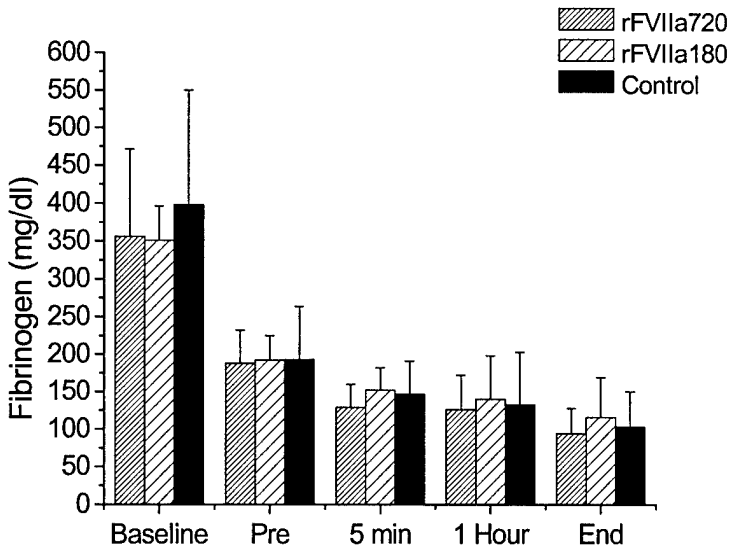
Figure 5. D-dimers



Despite the observed elevations in TATs and d-dimers, there was no decrease in either fibrinogen levels (Figure 6) or platelets in the treatment groups as compared to the control group. This suggests that rFVIIa did not induce the syndrome of disseminated intravascular coagulation or activate systemic coagulation. The elevations of TATs and

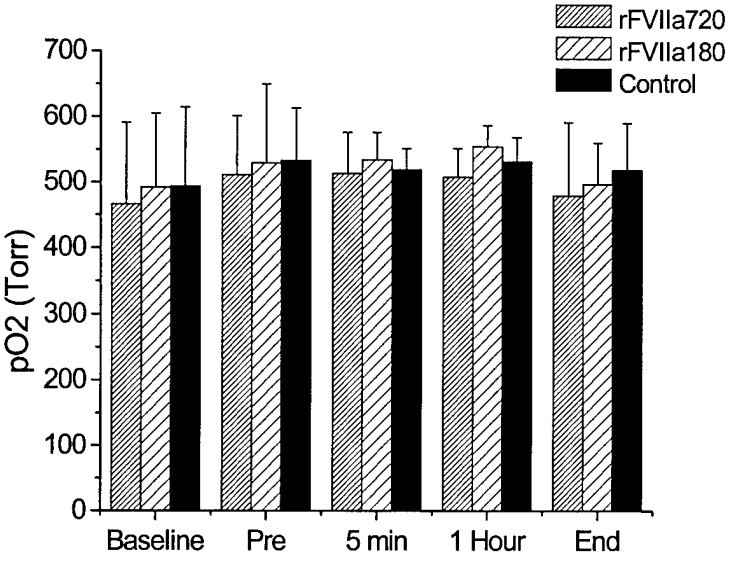
d-dimers are hypothesized to be secondary to increased activation of localized clotting activity at the site of injury. It should be noted that a human latex agglutination test was used to measure d-dimers. The validity of using human antibodies to detect pig d-dimers is unknown.

Figure 6. Fibrinogen levels.

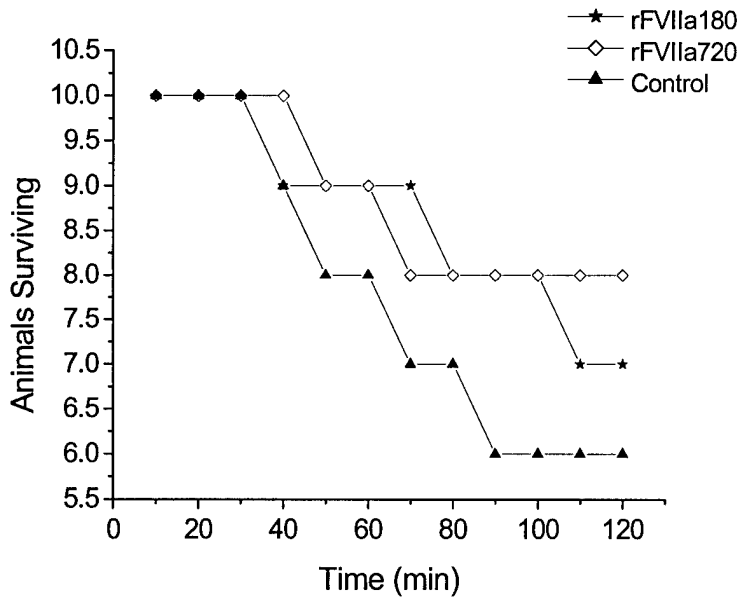


The prior study from our laboratory showed a trend toward increased pulmonary capillary microthrombosis in animals receiving rFVIIa. Histology studies from this study have not yet been concluded, however there was no evidence of decreased oxygenation in animals receiving rFVIIa. (Figure 7)

Figure 7. Oxygenation over the course of the study.



Survival during the 2 hours study was 6 out of 10 in the control group, 7 out of 10 in the 180 group and 8 out of 10 in the 720 group. There was no statistical difference in overall survival or mean survival time between the 3 groups. (Figure 8)



KEY RESEARCH ACCOMPLISHMENTS:

- Recombinant Factor VIIa reduces blood loss in hypothermic, dilutionally coagulopathic pigs with Grade V liver injuries when used as an adjunct to liver packing.
- Quadrupling the dose of rFVIIa does not increase its efficacy or alter its physiologic effects in any of the measured parameters.
- The infusion of rFVIIa resulted in a significant decrease in the prothrombin time but only in the initial period after treatment. Prothrombin times were not significantly different at 1 hour or the end of the study.
- The infusion of rFVIIa results in the elevation of thrombin antithrombin complexes and d-dimers in severely injured animals. However, there is no evidence of associated disseminated intravascular coagulation.

REPORTABLE OUTCOMES:

This research was presented at the 2001 Advanced Technology Applications to Combat Casualty Care conference in Ft. Walton Beach, Florida. This work has also been presented at the Eastern Association for the Surgery of Trauma meeting, held January 17 – 19, 2002 in Orlando, Florida. The work was published in the August 2002 version of the Journal of Trauma. A reprint of the publication is attached to this report.

CONCLUSIONS:

This work confirms that rFVIIa is effective as adjunctive therapy in a severe liver injury model in hypothermic, dilutionally coagulopathic pigs. This work is complementary to our prior study which showed no effect of rFVIIa in non-coagulopathic pigs with the same liver injury when the drug was used as sole therapy.[1] Combining the data from the 2 studies suggests that rFVIIa should be used as an adjunct to standard surgical therapy to control the bleeding of coagulopathy and hypothermia.

Increasing the dose of rFVIIa by 4 times did not increase its efficacy. This is most likely explained by the mechanism of action of the drug. Factor VIIa functions by binding exposed and activated tissue factor. It is likely that the dose of 180 μg/kg was adequate to saturate the available exposed and activated tissue factor. Therefore, increasing the dose of the drug had no additional effect.

Multiple avenues for future study exist. This work, in conjunction with prior work, establishes the efficacy of the drug in a trauma model. Questions, which remain to be answered, include the proper dosing of the drug. The fact that prothrombin times in our study were only significantly different between treatment and control at 5 minutes and not at 1 hour or the end of the study raises the question if repeat dosing or a continuous drip would improve the efficacy of the drug.

In addition to dosing issues, safety concerns still need to be addressed. Blunt trauma is associated with exposure and activation of tissue factor. Elevated tissue factor levels have been associated with the development of the Adult Respiratory Distress Syndrome in trauma patients.[6] We propose a series of studies utilizing well-established ARDS models to determine if the infusion of rFVIIa exacerbates the condition.

The first model was described by Hardaway and involves blunt soft tissue injury to the pig thigh.[7,8] Repeated soft tissue trauma in pigs has been shown to be associated with ARDS and Multiple Organ Failure. This is hypothesized to be secondary to cellular injury with exposure and activation of tissue factor. We plan a prospective blinded study utilizing this model with a rFVIIa group and a control group. We will compare the groups for survival, onset of hypoxia and evidence of MOF as evidenced by liver function tests and renal function. Histology will also be compared.

A second model involving the creation of ARDS secondary to a unilateral pulmonary contusion induced with a captive bolt gun has been described.[9,10] We plan to utilize this model to compare a rFVIIa group and a control group for survival as well as onset of ARDS and MOF. In order to more accurately reflect the trauma scenario, these studies will be performed with and without uncontrolled hemorrhagic shock utilizing our Grade V liver injury.

The proposed safety studies in combination with our prior efficacy studies should provide adequate data to proceed with a high quality prospective randomized trial in human trauma patients. Our laboratory looks forward to participating in these future trials and expediting the process of making rFVIIa available to the injured soldier.

REFERENCES:

1. Schreiber MA, Holcomb JB, Hedner U, Brundage SI, Macaitis J, Meng ZH, Aoki N, Tweardy D, Hoots K. The Effect of Recombinant Factor VIIa on Non-coagulopathic Pigs with Grade V Liver Injuries. Supplement to The Journal of Thrombosis and Haemostasis, Abstract OC 1762, July 2001 (ISSN 0340-6245)
2. Moore EE, Cogbill TH, Jurkovich GJ, et al. Organ Injury Scaling: Spleen and Liver (1994 revision). Journal of Trauma. 1995;38:323-324.
3. Holcomb JB, Pusateri AE, Harris RA, et al. Effect of Dry Fibrin Sealant Dressings vs. Gauze Packing on Blood Loss in Grade V Liver Injuries in Resuscitated Swine. Journal of Trauma. 1999;46:49-57.
4. Holcomb JB, Pusateri AE, Harris RA, et al. Dry Fibrin Sealant Dressings Reduce blood Loss, Resuscitation Volume and Improve Survival in Hypothermic Coagulopathic Swine with Grade V Liver Injuries. Journal of Trauma. 1999;47:233-242.
5. Martinowitz U, Holcomb JB, Pusateri AE, et al. Intravenous rFVIIa Administered for Hemorrhage Control in Hypothermic Coagulopathic Swine with Grade V Liver Injuries. Journal of Trauma. 2001;50:721-729.
6. Gando S, Nanzak S, Morimoto Y, et al. Systemic activation of Tissue Factor Dependent Coagulation Pathway in Evolving Acute Respiratory Distress Syndrome in Patients with Trauma and Sepsis. Journal of Trauma. 1999;47:719-723.
7. Hardaway RM, Williams CH, Marvasti M, Farias M, et al. Prevention of Adult Respiratory Distress Syndrome with Plasminogen Activator in Pigs. Critical Care Medicine. 1990;18:1413-1418.
8. Hardaway RM, Williams CH. Prevention of Multiple Organ Failure with Plasminogen Activator. Current Therapeutic Research. 1991;49:711-721.
9. Davis KA, Troke MS, Fabian TC, Croce MA, Proctor KG. Early Mediators in the Secondary Injury that Develops after Unilateral Pulmonary contusion. 1998;45:200.
10. Davis KA, Fabian TC, Croce MA, Proctor KG. Prostanoids: Early Mediators in the Secondary Injury that Develops after Unilateral Pulmonary Contusion. Journal of Trauma. 1999;824-832.

The Effect of Recombinant Factor VIIa on Coagulopathic Pigs with Grade V Liver Injuries

Martin A. Schreiber, MD, FACS, John B. Holcomb, MD, FACS, Ulla Hedner, MD, Susan I. Brundage, MD, FACS, Joseph M. Macaitis, BS, and Keith Hoots, MD

Background: **Recombinant factor VIIa (rFVIIa) has been used to decrease bleeding in a number of settings including hemophilia, liver transplantation, intractable bleeding, and cirrhosis. Experience in the trauma setting is limited. This study was performed to determine whether rFVIIa would reduce bleeding after a grade V liver injury in hypothermic, dilutionally coagulopathic pigs when used as an adjunct to abdominal packing and to determine whether increasing the dose of the drug increased its hemostatic efficacy.**

Methods: **Thirty animals were randomized to receive 180 μg/kg of rFVIIa, 720 μg/kg of rFVIIa, or vehicle buffer control. After laparotomy and splenectomy, animals underwent a 60% blood volume isovolemic exchange transfusion with 5% human albumin. The animals' temperature was maintained at 33°C and a standardized grade V liver injury was made with a liver clamp. Thirty seconds after injury, the abdomen was packed with laparotomy sponges, resuscitation was initiated, and blinded therapy was given. Animals were resuscitated to their baseline mean arterial pressure and the study was continued for 2 hours. Serial coagulation parameters were measured at the temperature they were drawn. After the study period, surviving animals were killed, posttreatment blood loss was measured, and an autopsy was performed.**

Results: **Ten animals were randomized to each group. After administration of study drug, factor VII clotting activity (FVII:C) was higher in the 720-μg/kg group than in the 180-μg/kg group ($p < 0.01$). FVII:C was higher in both treatment groups than in the control group ($p < 0.01$). The mean prothrombin time was shorter in the treatment groups than in the control group ($p < 0.05$). Mean arterial pressure was lower in the control group than in the treatment groups throughout the study ($p < 0.01$). Mean blood loss was less in the treatment groups than in the control group ($p = 0.03$). Mortality was not different between groups. There were no differences between the groups that received rFVIIa in any measured parameters except for FVII:C. Liver injuries were similar between groups and there was no evidence of microthrombosis on lung histology.**

Conclusion: **rFVIIa reduces blood loss in hypothermic, dilutionally coagulopathic pigs with grade V injuries when used as an adjunct to packing. Increasing the dose does not enhance the hemostatic effect.**

Key Words: **Recombinant factor VIIa, Liver injury, Swine, Hemorrhage, Hypothermia, Coagulopathy.**

J Trauma. 2002;53:252–259.

Recombinant factor VIIa (rFVIIa) has been described as a universal hemostatic agent.[1] It was originally designed to treat hemophiliacs with inhibitors to factor VIII and factor IX,[2,3] and it is approved by the Food and Drug Administration for that purpose. Successful use of the drug in patients with platelet disorders, cirrhosis, intractable bleeding from various causes, liver transplantation, and cardiac surgery has also been published.[4–11]

The published uses of rFVIIa in the clinical trauma setting have been limited, and consist of a case report and a small, uncontrolled case series. The case report involved an Israeli soldier who sustained a high-velocity rifle injury to his inferior vena cava. The patient appeared to be moribund despite maximum standard efforts, but intravenous infusion of rFVIIa resulted in arrest of blood loss and the patient survived.[12] The case series consisted of seven massively injured patients whose median transfusion requirement was 40 units. rFVIIa was used as an adjunct to standard surgical techniques. The administration of rFVIIa resulted in correction of coagulopathy and cessation of diffuse bleeding in all of the patients. Three of the seven patients died from reasons other than bleeding or thromboembolism.[13]

The use of rFVIIa in pigs with grade V liver injuries has also been described. When used as sole therapy in warm noncoagulopathic animals, a dose of 150 μg/kg has been shown to be ineffective in decreasing blood loss.[14] However, in a small series of 10 hypothermic and diluted animals with the same injury, 180 μg/kg of rFVIIa was shown to significantly reduce blood loss as compared with normal saline

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control when used as an adjunct to abdominal packing.[15] The effect of dose on hemostatic efficacy in the pig liver injury model has not been studied. This study was performed to confirm the efficacy of rFVIIa in reducing blood loss from grade V liver injuries in cold coagulopathic pigs when used as an adjunct to packing and to determine whether quadrupling the dose of the drug would increase its efficacy.

MATERIALS AND METHODS

Thirty Yorkshire crossbred swine of both sexes, weighing approximately 30 kg, were used. All animals were free of disease and in apparent excellent health. Animals were allowed free access to water and to a commercial laboratory swine food. Food was withheld the night before the study. All animals were maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and all experimental manipulations were performed in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals*. The protocol was approved by the Institutional Animal Care and Use Committee at Baylor College of Medicine.

The swine were anesthetized with an intramuscular injection of 4.4 mg/kg of Telezol. The animals were also given 0.25 mg/kg of glycopyrrolate intramuscularly. They were then intubated with a 7-mm Mallinckrodt endotracheal tube and placed on mechanical ventilation with settings of 10 mL/kg tidal volume, a respiratory rate of 12 to 14 breaths/min, and 100% oxygen. Respiratory rate and tidal volume were adjusted to maintain the P_{CO_2} close to 40 mm Hg. Anesthesia was maintained using 2% isoflurane and an esophageal temperature probe was placed.

Once the animals were fully anesthetized, cutdowns were performed and polyethylene tubing was placed in the left external jugular vein and left common carotid artery. The venous line was used for study drug infusion and fluid resuscitation. The arterial line was used for continuous blood pressure monitoring and blood sampling. Mean arterial pressure, systolic pressure, diastolic pressure, and heart rate were recorded and averaged every 10 seconds using a digital data collection system with a blood pressure analyzer (Micro-Med, Inc., Louisville, KY).

The animals underwent midline laparotomy, Foley catheter placement, and splenectomy. The spleen was weighed and lactated Ringer's (LR) solution at room temperature was infused at 100 mL/min to replace three times the spleen weight. After splenectomy and spleen replacement, there was a 15-minute stabilization period. Animals then underwent an isovolemic exchange transfusion with 5% human albumin at room temperature. An estimated 60% of the animal's blood volume was removed via controlled hemorrhage from the carotid arterial line. The following equation was used: blood volume (mL/kg) = $161.4751(\text{body weight}^{-0.2197})$.[16] The animal's temperature was then standardized to an esophageal temperature of 33°C. This was done by lavaging the abdomen with room-temperature LR solution. This model for hypothermia and coagulopathy is a modification of the model that was developed by Holcomb et al. to test the efficacy of a dry fibrin sealant dressing, and it is designed to reflect the scenario of massive blood loss and resuscitation seen in severely injured patients.[17]

Preweighed laparotomy pads were placed in both gutters and the pelvis to facilitate blood collection. A standardized grade V liver injury was made with a specially designed liver clamp. For the purposes of this model, a grade V injury is defined as an injury to a central hepatic vein. This is consistent with the definition of a grade V injury as indicated by the American Association for the Surgery of Trauma Organ Injury Scaling system.[18]

The clamp was positioned in the middle of the liver, placing the right hepatic vein, left hepatic vein, and portal vein at risk for injury. Blood loss was collected by suction. Thirty seconds after injury, blinded therapy consisting of either 180 μg/kg of rFVIIa, 720 μg/kg of rFVIIa, or the equivalent amount of buffer solution was infused. Simultaneously, the liver injury was packed with laparotomy sponges, and resuscitation with LR solution at 100 mL/min was initiated. The temperature of the resuscitation fluid was varied to maintain a core temperature 33°C. The abdomen was then closed. Animals were resuscitated to their baseline mean arterial pressure (MAP). Pretreatment blood loss was calculated as the sum of the volume of blood suctioned and the difference in weight of the preweighed laparotomy pads before and after bleeding.

The study was continued for 2 hours from the time of injury. During this time period, LR solution was given as needed to maintain the baseline MAP. The core temperature was maintained at 33°C by varying the fluid temperature and by using a Bair Hugger warming system (Augustine Medical, Inc., Eden Prairie, MN). Time of death was recorded for those animals that did not survive the 2-hour study period.

After 2 hours, the animals were killed and the abdomen was opened. Free blood in the abdomen was suctioned and the laparotomy sponges were weighed. Posttreatment blood loss was calculated as the sum of the volume of blood suctioned and the difference in weight of the laparotomy sponges from before placement to the end of the study. A necropsy of the liver was performed to ensure that injuries were comparable between groups and the left lung was removed for histology.

Laboratory studies were drawn at baseline (after splenectomy and spleen replacement), just before injury, 5 minutes after injury, 1 hour after injury, and at the end of the study. Laboratory studies included arterial blood gas, complete blood count, factor VII clotting activity (FVII:C), prothrombin time (PT), partial thromboplastin time, fibrinogen, and thrombin-antithrombin complexes (TATs). Coagulation studies were performed at the temperature at which they were drawn. D-dimers were not used in this study because a reliable test for pigs has not yet been developed and verified.

Table 1 Comparison of Mean Weight, Mean Injury Temperature, Mean Number of Vessels Injured, and Mean Fluid Resuscitation between Groups

	180 μg/kg	720 μg/kg	Control	p Value
Mean weight (kg)	31.4 ± 2.8	31.7 ± 3.3	31.6 ± 3.5	NS
Mean injury temperature (°C)	33.1 ± 0.4	33.3 ± 0.2	33.2 ± 0.2	NS
Mean vessels injured	2 ± 0.8	2.1 ± 0.6	2.1 ± 0.7	NS
Mean fluid resuscitation (mL)	2,717 ± 3,324	2,734 ± 2,139	4,514 ± 3,566	0.2

Lung histology was examined by an independent blinded pathologist. Hematoxylin and eosin staining as well as immunostaining for fibrin were performed. Immunostains were prepared using a two-layered method. After antigen retrieval using 1% protease, rabbit anti-human fibrinogen antibody (DAKO A0080 was applied as primary antibody and goat anti-rabbit antibody labeled with horseradish peroxidase (Jackson 111-035-003) as secondary antibody. Vector red served as chromogene and a positive control section was included. As a negative control, an adjacent section was stained as above without the addition of the primary antibody.

Statistical Analysis

The Student's t test was used to compare the means of continuous variables. Dichotomous data were analyzed with the χ^2 test. If the value of a categorical variable in any cell was less than 5, Fisher's exact test was used. Statistical analysis was performed using commercially available software from Stata Corporation (College Station, TX). Statistical significance was defined as a value of $p < 0.05$.

RESULTS

Ten animals were randomized to each of the three groups. Mean weight, temperature, number of vessels injured, and fluid resuscitation are shown with their standard deviations in Table 1. Weight and temperature were similar between groups, as was the severity of injury. Animals in the control group received approximately twice as much resuscitation fluid as animals in the two treatment groups. However, because of large standard deviations, these differences were not statistically significant.

Pretreatment blood loss was nearly identical among the three groups. Mean blood loss after treatment was 2,187 mL in the control group versus 1,085 mL in the 180-μg/kg group and 1,086 mL in the 720-μg/kg group. This difference was significant at the $p = 0.03$ level when the two treatment groups were combined and compared with the control group. There was no difference in blood loss between the two treatment groups (Fig. 1).

The mean arterial pressures of the three groups are shown in Figure 2. The 60% isovolemic hemodilution resulted in marked hypotension with a mean preinjury MAP for all animals of 41.7 ± 3.7 mm Hg. After injury, the nadir MAP occurred at a mean of 4.5 ± 3.1 minutes and the average MAP dropped by 37% ± 16%. These values were not different between groups. Throughout the remainder of the study, the mean MAP of the control group was significantly lower than the treatment groups ($p < 0.05$). There was no difference between the two treatment groups.

After treatment, FVII:C increased by 340-fold over baseline in the 720-μg/kg group and by 103-fold in the 180-μg/kg group ($p < 0.01$). FVII:C remained significantly greater in the 720-μg/kg group than in the 180-μg/kg group throughout the remainder of the study and significantly higher in the 180-μg/kg group than in the control group (Fig. 3).

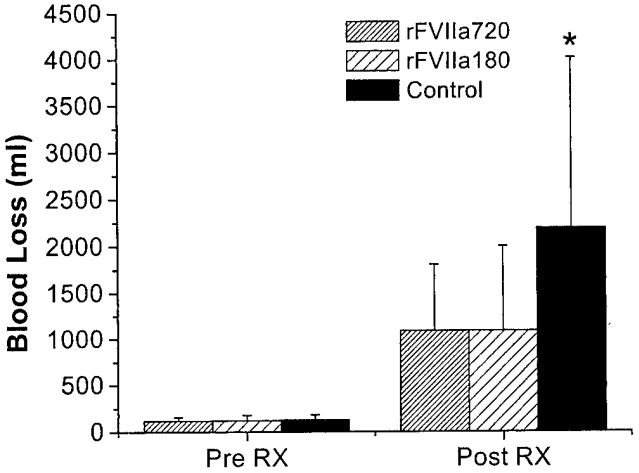

Fig. 1. *Blood loss before and after treatment. *p = 0.03 for the combined treatment groups versus the control group.*

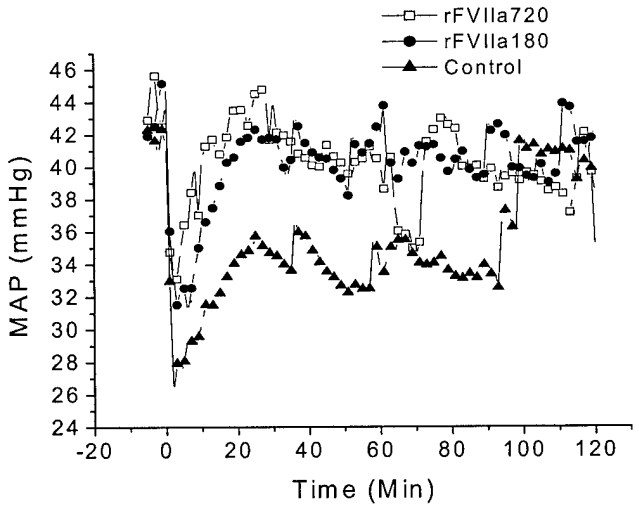

Fig. 2. *Serial mean arterial pressures over the course of the study.*

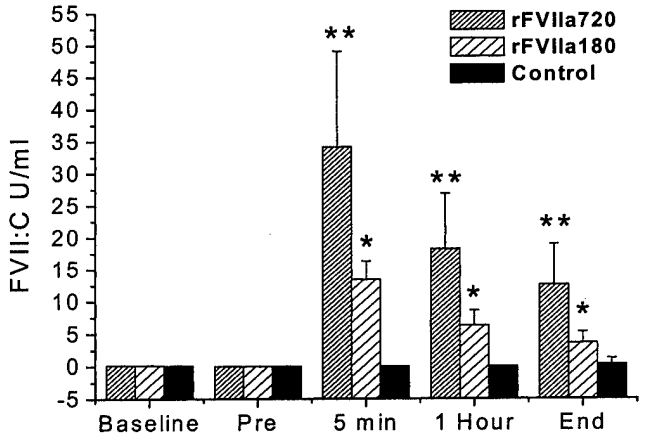

Fig. 3. *Mean factor VII clotting activity (FVII:C). *p < 0.01 for 180 μg/kg versus control; **p < 0.01 for 720 μg/kg versus 180 μg/kg and control.*

Mean PTs measured over the course of the study are shown in Figure 4. Prothrombin times were measured at the temperature at which they were drawn. After treatment, there was a significant reduction in the PTs in the two treatment groups as compared with the control group. This difference was no longer statistically significant 1 hour after injury or at the end of the study. The PT in the control group at the end of the study was significantly greater than the preinjury PT, probably representing ongoing hemodilution from increased blood loss resulting in decreased MAP, thus necessitating increased resuscitation. Prothrombin values at the end of the study in the treatment groups were not significantly different compared with preinjury values. Partial thromboplastin times were not significantly different over the course of the study between groups.

TATs are compared serially between groups in Figure 5. TATs increased significantly during the observation period in all groups. The groups that received rFVIIa exhibited signif-

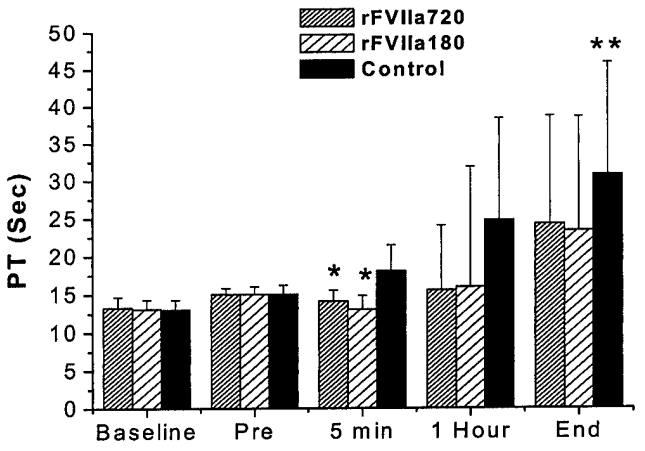

Fig. 4. *Mean prothrombin times. *p < 0.05 for 720 μg/kg versus control and for 180 μg/kg versus control; **p < 0.01 for control at end versus preinjury control.*

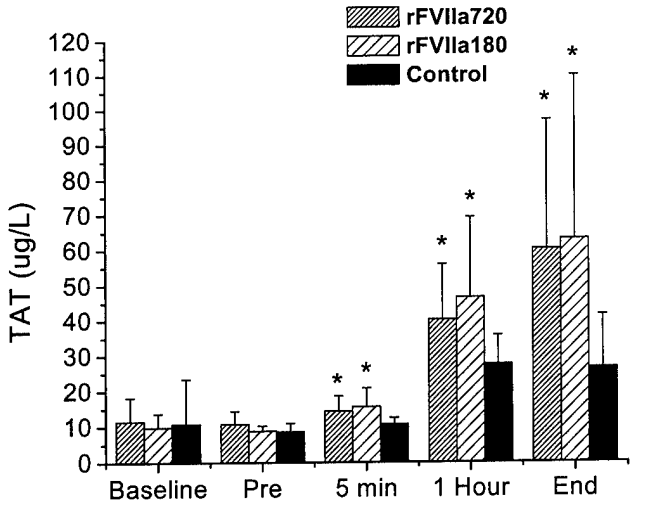

Fig. 5. *Thrombin-antithrombin complexes (TATs). *p < 0.05 for 720 μg/kg versus control and for 180 μg/kg versus control.*

icantly higher TATs compared with the control group at all time points after treatment, including the 5-minute postinfusion sampling time. TATs were not different between the two treatment groups.

Fibrinogen levels (Fig. 6) and platelet counts (Fig. 7) decreased significantly over the course of the study in all three groups, most likely as a result of dilution. There was no difference in these parameters between groups at any time point. Because of concerns of rFVIIa causing abnormal pulmonary microthrombosis, oxygen tension was measured throughout the study. There was no difference in oxygenation between groups or within groups over the course of the study. Similarly, lung histology revealed no evidence of premorbid microthrombosis or abnormal clotting of the pulmonary vasculature.

Survival during the 2-hour study was 6 of 10 in the control group, 7 of 10 in the 180-μg/kg group, and 8 of 10 in

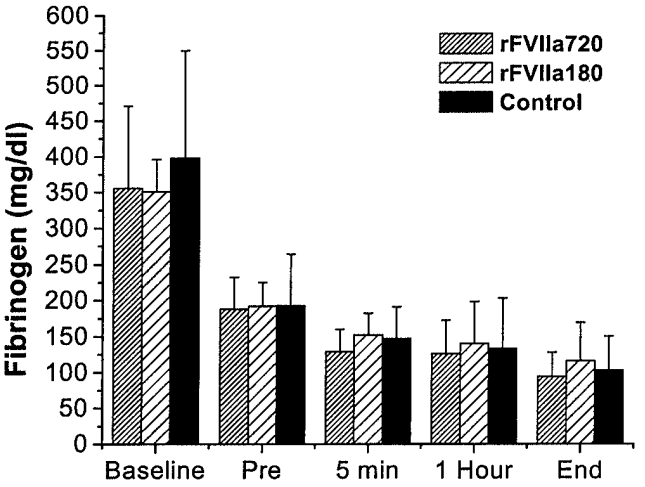

Fig. 6. *Mean fibrinogen levels compared between groups.*

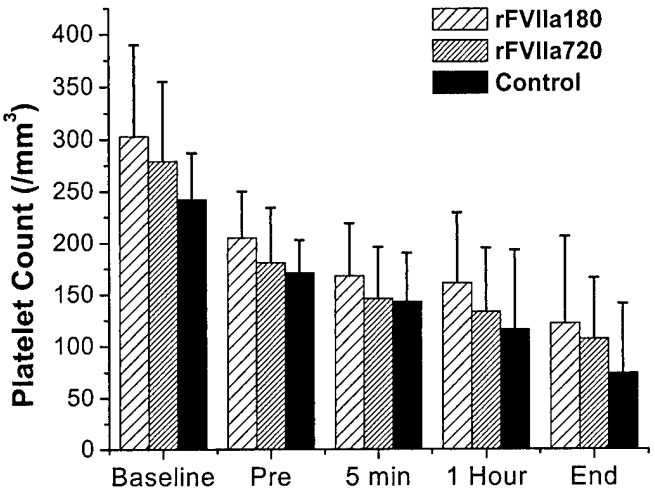

Fig. 7. *Mean platelet counts compared serially over the course of the study.*

the 720-μg/kg group. There was no difference in overall survival or mean survival time between the three groups (Fig. 8).

DISCUSSION

This study confirms the findings of Martinowitz et al. that rFVIIa reduces blood loss in animals with grade V liver injuries when used as an adjunct to liver packing.[15] This study differed from the study by Martinowitz et al. because 5% human albumin was used for the 60% isovolemic hemodilution as opposed to hetastarch. Five percent human albumin was used to avoid the coagulopathy seen with the high-molecular-weight hetastarch that is commonly used in the United States.[19–21]

The hemostatic effect of rFVIIa was not enhanced by quadrupling the dose of the drug despite evidence of the increased dose on the basis of elevated FVII:C. Mean arterial

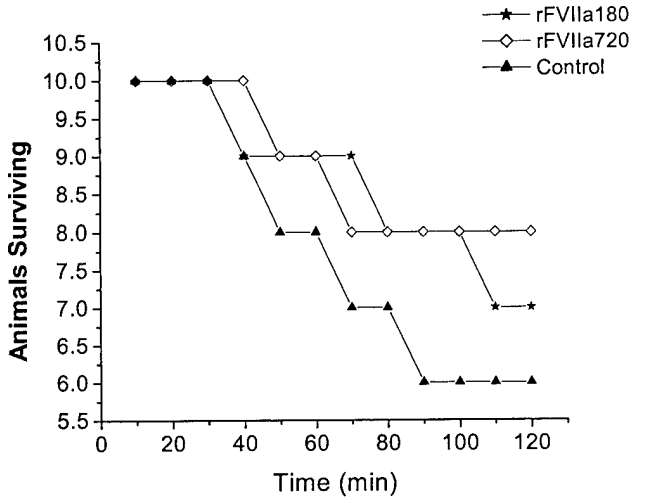

Fig. 8. *Survival curves.*

pressure and other coagulation parameters were also not different between the two treatment groups. The absence of an added effect in the higher dose group may be related to the mechanism of action of the drug. Injury results in exposure and deencryption of tissue factor at the site of injury, which then combines with FVIIa. The FVIIa–tissue factor complex then activates FIX and FX, resulting ultimately in thrombin production and cleavage of fibrinogen to fibrin. Thrombin formation also results in platelet membrane changes, causing negatively charged phospholipids to be exposed. This negatively charged surface is the template for full thrombin generation also involving FVIII and FIX. The absence of an increased hemostatic effect of the higher dose of 720 μg/kg suggests that the system was already saturated by the 180-μg/kg dose, rendering the increased dose ineffective.

Our prior work revealed no hemostatic effect of rFVIIa when it was used as a sole agent in warm, noncoagulopathic animals with the same grade V injury described in this study.[14] Three hypothetical explanations for a lack of effect were surmised. These included use of the drug as the sole hemostatic agent, relative hypotension of the cold, diluted animals, and suboptimal dosing of the drug in the pig model. The results of this study suggest that in coagulopathic pigs with severe venous and parenchymal liver injuries, 180 μg/kg is an adequate dose of the drug when it is used as an adjunct. Use of the drug as sole therapy in this massive liver injury and the normotensive state of the warm nondiluted animals remain likely explanations for the lack of an effect in the prior study.

Thrombin-antithrombin complexes are an indirect measure of thrombin generation. As thrombin is formed, antithrombin III complexes with it as part of a negative-feedback loop. TATs increased significantly in all three groups over the course of the study. This indicates that TATs were formed independent of rFVIIa. TATs were significantly elevated in the treatment groups relative to the control group. Despite the fact that elevation of TATs has been associated with thrombogenic situations and disseminated intravascular coagulation,[22–24] the absence of decreased platelet counts and fibrinogen levels in the treatment groups provides strong evidence that rFVIIa did not induce any systemic activation of the coagulation system or disseminated intravascular coagulation. The data suggest that rFVIIa resulted in enhanced thrombin formation only at the site of injury. The fact that TAT formation was not dose dependent is in accordance with other data from this study indicating that the system was saturated by the 180-μg/kg dose.

Despite the use of a supratherapeutic dose of rFVIIa, there was no evidence of pathologic coagulation by laboratory parameters or lung histology. These findings are consistent with prior studies in humans and animals.

The current study represents the largest trial reporting the effects of rFVIIa in pigs and the first trauma study to examine the effects of dose escalation. The study is weakened by the large variability in blood loss and resuscitation inherent in

uncontrolled hemorrhage models. This resulted in variable dilutional effects, explaining the large standard deviations seen in the coagulation parameters, especially at 1 hour and at the end of the study. Because of large standard deviations, the treatment groups were combined to show a decrease in post-treatment blood loss compared with the control group. This was warranted because there was no measurable physiologic or laboratory difference between the treatment groups except for FVII:C, which confirmed that different doses were given.

This study contributes to the increasing body of animal and human data suggesting that rFVIIa is efficacious and safe in the setting of trauma and hemorrhagic shock. Adequate preliminary data exist to support a randomized prospective study comparing rFVIIa to placebo as adjunctive therapy in human trauma patients with hemorrhagic shock, ongoing bleeding, and coagulopathy.

ACKNOWLEDGMENT

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REFERENCES

1. Hedner U. Recombinant activated factor VII as a universal haemostatic agent. *Blood Coagul Fibrinolysis.* 1998;9(suppl 1):S147–S152.
2. Hedner U. Treatment of patients with factor VIII, and factor IX inhibitors with special focus on the use of recombinant factor VIIa. *Thromb Haemost.* 1999;82:531–539.
3. Shapiro AD, Gilchrist GS, Hoots WK, Cooper HA, Gastineau DA. Prospective, randomized trial of two doses of rFVIIa (NovoSeven) in haemophilia patients with inhibitors undergoing surgery. *Thromb Haemost.* 1998;80:773–778.
4. Bernstein DE, Jeffers L, Erhardtsen E, et al. Recombinant factor VIIa corrects prothrombin time in cirrhotic patients: a preliminary study. *Gastroenterology.* 1997;113:1930–1936.
5. Papatheodoridis GV, Chung S, Keshav S, Pasi J, Burroughs AK. Correction of both prothrombin time and primary haemostasis by recombinant factor VII during therapeutic alcohol injection of hepatocellular cancer in liver cirrhosis. *J Hepatol.* 1999;31:747–750.
6. Kristensen J, Killander A, Hippe E, et al. Clinical experience with recombinant factor VIIa in patients with thrombocytopenia. *Haemostasis.* 1996;26(suppl 1):159–164.
7. Tengborn L, Petruson B. A patient with Glanzmann's thrombasthenia and epistaxis successfully treated with recombinant factor VIIa. *Thromb Haemost.* 1996;75:981–982.
8. Douri MA, Shafi T, Khudairi DA, et al. Effect of the administration of recombinant activated factor VII (rFVIIa; NovoSeven) in the management of severe uncontrolled bleeding in patients undergoing heart valve replacement surgery. *Blood Coagul Fibrinolysis.* 2000; 11(suppl 1):S121–S127.
9. White B, Ravi N, McHale J, et al. Successful use of recombinant FVIIA (Novoseven) in the management of intractable post surgical intra-abdominal haemorrhage. *Br J Haematol.* 1999;107:677–678.
10. Vlot AJ, Ton E, Mackaay AJ, Kramer MH, Gaillard CA. Treatment of a severely bleeding patient without preexisting coagulopathy with activated recombinant factor VII. *Am J Med.* 2000;108:421–423.
11. Kalicinski P, Kaminski A, Drewniak T. Quick correction of hemostasis in two patients with fulminant liver failure undergoing liver transplantation by recombinant activated factor VII. *Transplant Proc.* 1999;31:378–379.
12. Kenet G, Walden R, Eldad A, Martinowitz U. Treatment of traumatic bleeding with recombinant factor VIIa. *Lancet.* 1999; 354:1879.
13. Martinowitz U, Kenet G, Segal E, et al. Recombinant activated factor VII for adjunctive hemorrhage control in trauma. *J Trauma.* 2001;51:431–439.
14. Schreiber MA, Holcomb JB, Hedner U, et al. The effect of recombinant factor VIIa on non-coagulopathic pigs with grade V liver injuries [abstract]. *Thromb Haemost.* 2001;86(suppl):Abstract OC 1762.
15. Martinowitz U, Holcomb JB, Pusateri AE, et al. Intravenous rFVIIa administered for hemorrhage control in hypothermic coagulopathic swine with grade V liver injuries. *J Trauma.* 2001;50:721–729.
16. Bush JA, Jensen WN, Cartwright GE, Wintrobe MM. Blood volume studies in normal and anemic swine. *Am J Physiol.* 1955;181:9–14.
17. Holcomb JB, Pusateri AE, Harris RA, et al. Dry fibrin sealant dressings reduce blood loss, resuscitation volume and improve survival in hypothermic coagulopathic swine with grade V liver injuries. *J Trauma.* 1999;47:233–242.
18. Moore EE, Cogbill TH, Jurkovich GJ, Shackford SR, Malangoni MA, Champion HR. Organ injury scaling: spleen and liver (1994 revision). *J Trauma.* 1995;38:323–324.
19. Stump DC, Strauss RG, Henriksen RA, Petersen RE, Saunders R. Effects of hydroxyethyl starch on blood coagulation, particularly factor VIII. *Transfusion.* 1985;25:349–354.
20. Jamnicki M, Bombeli T, Burkhardt S, et al. Low- and medium-molecular-weight hydroxyethyl starches: comparison of their effect on blood coagulation. *Anesthesiology.* 2000;93:1231–1237.
21. Franz A, Braunlich P, Gamsjager T, Felfering M, Gustorff B, Kozek-Langenecker SA. The effects of hydroxyethyl starches of varying molecular weights on platelet function. *Anesth Analg.* 2001; 92:1402–1407.
22. Takahashi H, Tatewaki W, Wada K, Hanano M, Shibata A. Thrombin vs. plasmin generation in disseminated intravascular coagulation associated with various underlying disorders. *Am J Hematol.* 1990;33:90–95.
23. Schmidt B, Vegh P, Johnston M, Andrew M, Weitz J. Do coagulation screening tests detect increased generation of thrombin and plasmin in sick newborn infants? *Thromb Haemost.* 1993; 69:418–421.
24. Gouin-Thibault I, Achkar A, Samama MM. The thrombophilic state in cancer patients. *Acta Haemotol.* 2001;106:33–42.

DISCUSSION

Dr. Steven N. Vaslef (Durham, North Carolina): This is a follow-up study to one in which this group demonstrated that a dose of 180 μg/kg of recombinant factor VIIa significantly decreased bleeding as compared with normal saline control when used as an adjunct to abdominal packing in a liver injury model in swine. The present study was performed to confirm the efficacy of recombinant factor VIIa in reducing blood loss from grade V liver injuries in cold coagulopathic pigs when used as an adjunct to packing in grade V liver injuries. It also seeks to determine the optimal dose of recombinant factor VIIa after such an injury. The article is well written, easy to follow, and a pleasure to read.

I have several comments and questions. First, there are two possible mechanisms of action postulated to explain how recombinant factor VIIa promotes coagulation. One is a tissue factor–dependent process in which recombinant factor VIIa is combined with tissue factor to generate factors IXa and Xa to ultimately form a clot.